

CLAIMS:

1. A method for the production of modified endosperm, which comprises the step of transforming a plant, or plant propagating material, with a nucleic acid molecule comprising one or more regulatory sequences capable of directing expression in the male or female germ line and/or gametes of the resultant plant, and one or more sequences whose expression or transcription product(s) is/are capable of modulating genomic imprinting.
- 10 2. A method for the production of modified endosperm, which comprises the step of transforming a plant, or plant propagating material, with a nucleic acid molecule comprising one or more regulatory sequences capable of directing expression within the developing gynoecium, especially the cell lineage that gives rise to or comprises the female germ line (megasporocyte tissue), within the ovule of the resultant plant,
- 15 and one or more sequences whose expression or transcription product(s) is/are capable of modulating genomic imprinting.
- 20 3. A method for the production of modified endosperm which comprises the step of transforming a plant, or plant propagating material, with a nucleic acid molecule comprising one or more regulatory sequences capable of directing expression within the developing stamen, especially the cell lineage that gives rise to or comprises the male germ line (microsporocyte tissue) of the resultant plant and one or more sequences whose expression or transcription product(s) is/are capable of modulating genomic imprinting.
- 25 4. A method as claimed in any one of claims 1 to 3 wherein the nucleic acid molecule comprises one or more sequences whose expression or transcription product(s) is/are associated with the formation and/or maintenance of genomic imprints.

30

5. A method as claimed in claim 4 wherein the nucleic acid molecule includes a sequence encoding a histone deacetylase, methyl cytosine binding protein or Sin 3 protein.

5 6. A method as claimed in any one of claims 1 to 5 wherein the nucleic acid
W 6/7 molecule includes a sequence of the FIE gene or FIS genes.

10 7. A method as claimed in any one of claims 1 to 6 wherein the nucleic acid
molecule comprises one or more sequences whose expression or transcription
product(s) is/are capable of altering the degree of methylation of nucleic acid.

15 8. A method for the production of modified endosperm, which comprises the step
of transforming a plant, or plant propagating material, with a nucleic acid molecule
comprising one or more regulatory sequences capable of directing expression in the
male or female germ line and/or gametes of the resultant plant, and one or more
sequences whose expression or transcription product(s) is/are capable of altering the
degree of methylation of nucleic acid.

20 9. A method for the production of modified endosperm, which comprises the step
of transforming a plant, or plant propagating material, with a nucleic acid molecule
comprising one or more regulatory sequences capable of directing expression within
the developing gynoecium, especially the cell lineage that gives rise to or comprises
the female germ line (megasporocyte tissue), within the ovule of the resultant plant,
and one or more sequences whose expression or transcription product(s) is/are capable
of altering the degree of methylation of nucleic acid.

25 10. A method for the production of modified endosperm which comprises the step
of transforming a plant, or plant propagating material, with a nucleic acid molecule
comprising one or more regulatory sequences capable of directing expression within
the developing stamen, especially the cell lineage that gives rise to or comprises the

male germ line (microsporocyte tissue) of the resultant plant and one or more sequences whose expression or transcription product(s) is/are capable of altering the degree of methylation of nucleic acid.

- 5 11. A method as claimed in any one of claims 1 to 10 wherein the one or more regulatory sequences comprise a promoter sequence, or regulatory sequences or fragments therefrom.
- 10 12. A method as claimed in any one of claims 1, 2, 4 to 9, or 11 wherein the promoter is derived from the *Arabidopsis* AGL5 gene, the *Petunia* FBP7, the *Petunia* FBP11 gene, the *Arabidopsis* BEL1 gene, the *Arabidopsis* MEDEA (*FIS1*) gene, the *Arabidopsis* FIS 2 gene, the *Arabidopsis* FIE (*FIS 3*) gene, orthologs/homologues of any of these genes from other species or any promoter that drives expression that is restricted to cells within the female reproductive organs that contribute to the female germ line, preferably promoters from gynoecium-specific genes that are first expressed during early gynoecium development, preferably before the differentiation of individual ovules, and which maintain their expression until ovule differentiation is complete.
- 15 13. A method as claimed in any one of claims 1, 3, 4 to 8, 10 or 11 wherein the promoter is derived from the *Arabidopsis* gene APETALA3, the *Arabidopsis* PISTILATTA gene, the *Arabidopsis* E2 gene, the *Arabidopsis* APG gene, homologues/orthologs of these genes from other species or any promoter that drives expression that is restricted to cells within the male reproductive organs that contribute to the male germ line, preferably promoters from stamen-specific genes that are first expressed during early stamen development, preferably before the differentiation of individual microsporocytes, and which maintain their expression until stamen differentiation is complete.
- 20
- 25

14. A method as claimed in any one of claims 1 to 13 wherein the size of the endosperm is altered.

5 15. A method as claimed in any one of claims 1 to 13 wherein development of the endosperm is altered.

16. A method as claimed in any one of claims 7 to 15 wherein the degree of nucleic acid methylation is increased.

10 17. A method as claimed in claim 16 wherein the nucleic acid molecule includes a sequence encoding a methylating enzyme such as Methylase 1, Methylase 1-like enzyme, Methylase 2 or Chromomethylase of *Arabidopsis*.

15 18. A method as claimed in any one of claims 7 to 15 wherein the degree of nucleic acid methylation is decreased.

19. A method as claimed in claim 18 wherein the nucleic acid molecule includes a sequence encoding a de-methylating enzyme such as de-methylase (= MeCP2-homologue) of *Arabidopsis*.

20 20. A method as claimed in claim 18 wherein reduction in nucleic acid methylation is achieved by down-regulation of one or more methylating enzymes present in the plant.

25 21. A method as claimed in claim 20 wherein the nucleic acid molecule includes a sequence encoding an antisense nucleic acid molecule, a full or partial copy of a methylating enzyme gene already present in the plant or sequence, or a sequence encoding a ribozyme.

22. A method as claimed in claim 21 wherein the nucleic acid molecule includes the sequence of the Met1as "gene".
- 5 23. An isolated or recombinant nucleic acid molecule, eg a DNA molecule, which comprises one or more regulatory sequences capable of directing expression in the male or female germ line and/or gametes of a plant and one or more sequences whose expression or transcription product(s) is/are capable of altering the degree of methylation of nucleic acid.
- 10 24. A nucleic acid molecule as claimed in claim 23 wherein the degree of nucleic acid methylation is decreased.
25. The use of a nucleic acid molecule as claimed in claim 24 as a barrier to hybridisation between plants.
- 15 26. The use as claimed in claim 25 wherein the plants are of the same species.
- 20 27. The use as claimed in claim 25 or claim 26 wherein the barrier results from failure in endosperm development.
28. The use of a nucleic acid molecule as claimed in claim 24 in overcoming a barrier to hybridisation between plants.
29. The use as claimed in claim 28 wherein the plants are of the same species.
- 25 30. The use as claimed in claim 28 or claim 29 wherein the barrier results from failure in endosperm development.

31. A nucleic acid molecule as claimed in claim 23 modified by any one or more of the features defined in any one of claims 12 to 22.
- 5 32. A nucleic acid molecule as claimed in any one of claims 23 to 31 which is in the form of a vector.
33. A plant cell including nucleic acid as defined in any one of claims 23 to 32.
- 10 34. A transgenic plant (or parts thereof such as propagating material) including nucleic acid as defined in any one of claims 23 to 33.
- 15 35. A method for modulating genomic imprinting in plants, which comprises the step of transforming a plant, or plant propagating material, with a nucleic acid molecule comprising one or more regulatory sequences capable of directing expression in the male or female germ line and/or gametes of the resultant plant, and one or more sequences whose expression or transcription product(s) is/are capable of altering the degree of methylation of nucleic acid.